

IN THE CLAIMS:

Please cancel pending claims without prejudice or disclaimer and examine newly presented claims 45-78.

Claims 1-44 (Cancelled)

45. (New) An isolated and purified endothelial cell growth factor obtained from bovine tissue which comprises a protein which is obtained from a culture of bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity.

46. (New) The isolated and purified bovine endothelial cell growth factor of Claim 45 which comprises an unglycosylated form of a glycoprotein obtained from a culture of bovine folliculi stellate cells.

47. (New) The isolated and purified bovine endothelial cell growth factor of Claim 45 wherein the folliculi stellate cells are derived from a bovine source which factor comprises at its N-terminus the amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-.

48. (New) An isolated and purified bovine endothelial cell growth factor obtained from bovine tissue which comprises a protein which is obtained from a culture of bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45 kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity.

49. (New) The isolated and purified bovine endothelial cell growth factor according to Claim 48 which comprises at its N-terminus the amino acid sequence <sup>1</sup>Ala-Pro-Met-Ala-Glu-

Gly-Gly-Gln-Gln-Lys-Pro-His-Glu.

50. (New) The isolated and purified bovine endothelial cell growth factor of Claim 49 which comprises at its N-terminus the amino acid sequence

Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-Val-Val-Lys-Phe-Met-Asp-Val-Tyr-Gln-.

51. (New) The isolated and purified bovine endothelial cell growth factor of Claim 45 having an N-terminus amino acid sequence of - .<sup>1</sup> Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His- Glu-Val-Val-Lys-Phe-Met-Asp-Val-Tyr-Gln-(Arg)-Ser-Phe-X-Arg-Pro-Ile-Glu-Thr-Leu-(Val)-X-Ile-X-(Gln)-Glu-Tyr-(Pro)-

wherein the amino acids in parenthesis are uncertain and the -X indicates an amino acid of unknown identity.

52. (New) The isolated and purified bovine endothelial cell growth factor according to Claim 45 which has a molecular weight of approximately 43Kd.

53. (New) The isolated and purified bovine endothelial cell growth factor according to Claim 52 which, under reducing conditions, forms two substantially homologous units, each unit having a molecular weight of about 23,000 daltons.

54. (New) An isolated and purified endothelial cell growth factor obtained from bovine tissue which comprises a protein which is obtained from a culture of bovine folliculi stellate cells and which is in the form of a dimeric protein having a molecular weight of approximately 43-45kd as determined by SDS PAGE, under nonreducing conditions which isolated factor has endothelial cell growth factor activity, which factor further comprises an internal amino acid sequence, obtainable upon tryptic digestion, which sequence comprises Ser-Phe-Cys-Arg-Pro-

Ile-Glu-Thr-Leu-Val-Asp-Ile-Phe-Gln-Glu-Tyr-Pro-Asp-Glu-Ile-; or Ser-Phe-Cys-Arg-Pro-Ile-Glu-Thr-Leu-Val-Asp-Ile-Phe-Gln-Glu-Tyr-Pro-Asp/Ile-Glu-.

55. (New) The pharmaceutical composition for use with a human being comprising the isolated and purified bovine endothelial cell growth factor of claim 48 and a pharmaceutically - acceptable carrier.

56. (New) The method of promoting the proliferation of bovine endothelial cells in vitro which comprises applying to an effective amount of an isolated and purified endothelial cell growth factor to endothelial cells of Claim 45.

57. (New) The method of Claim 56, wherein the endothelial cells are grown in cell culture.

58. (New) The pharmaceutical composition comprising the isolated and purified bovine endothelial cell growth factor of Claim 45 and a pharmaceutically-acceptable carrier.

59. (New) The pharmaceutical composition of Claim 58 wherein the carrier is a parenteral carrier.

60. (New) The pharmaceutical composition of Claim 58 which is adapted for wound healing in a human being by administering said growth factor to a human being at a concentration of between about 10 picogram/milliliter and about 500 picogram/milliliter.

61. (New) An isolated and purified bovine endothelial cell growth factor having wound healing activity which factor is obtained by a method of obtaining a substantially pure bovine endothelial cell growth factor, which method comprises:

(a) providing folliculi stellate cells from a bovine source containing levels of endothelial cell growth factor;

- (b) extracting a solubilized portion of said cells;
  - (c) partially purifying the endothelial cell growth factor from the extract using aqueous salt precipitation;
  - (d) fractionating the purified extract using affinity chromatograph employing heparin moieties linked to an insoluble support as the stationary phase and employing a step gradient mobile phase of increasing salt concentration;
  - (e) fractionating the extract using gel exclusion chromatography; and optionally
  - (f) purifying the extract using reverse-phase high pressure liquid chromatography.
62. (New) The bovine endothelial cell growth factor of Claim 45 for use in the treatment of wounds.
63. (New) The pharmaceutical composition comprising said bovine endothelial cell growth factor of Claim 60 for use in wound healing.
64. (New) The isolated bovine endothelial cell growth factor obtained by a process for producing bovine endothelial cell growth factor comprising, providing a replicable expression vector capable of expressing the DNA sequence encoding human endothelial cell growth factor in a suitable host, transforming said host to obtain a recombinant host and maintaining said recombinant host under conditions permitting expression of said endothelial growth factor-encoding cDNA sequence to produce the endothelial cell growth factor which is isolated.
65. (New) The isolated bovine endothelial cell growth factor obtained by said process of 64 wherein in the process said expression vector is selected from the group consisting of a bacteriophage and a plasmid.
66. (New) The bovine endothelial cell growth factor obtained by the process for

producing bovine endothelial cell growth factor comprising, providing a replicable expression vector capable of expressing the DNA sequence encoding bovine endothelial cell growth factor in a suitable host, transforming said host to obtain a recombinant host and maintaining said recombinant host, under conditions permitting expression of said endothelial growth factor - encoding cDNA sequence to produce the endothelial cell growth factor which is isolated .

67. (New) The isolated bovine endothelial cell growth factor obtained by the process of Claim 66 wherein in the process said expression vector is selected from the group consisting of a bacteriophage and a plasmid.

68. (New) A method of promoting the proliferation of human endothelial cells in vivo, which method comprises:

- (a) applying to endothelial cells a therapeutically effective amount of an isolated and purified endothelial cell growth factor wherein

said endothelial cell growth factor is a protein obtained from bovine folliculi stellate cells in the form of a dimeric protein having a molecular weight of approximately 43–45 kd which protein is obtained by SDS PAGE under non-reducing conditions, and

- (b) obtaining in vivo endothelial cell growth .

69. (New) The method of Claim 68 wherein in step (a) the protein further includes a pharmaceutically-acceptable carrier.

70. (New) The method of Claim 69 wherein said protein is present in between about 10 picrogram/ml and 500 picrogram/ml.

71. (New) The method of Claim 68 wherein said purified bovine endothelial cell growth factor forms two substantially homologous units each having a molecular weight of about 23 kd.

72. (New) The method of Claim 68 wherein for the isolated and purified bovine endothelial cell growth factor the folliculi stellate cells are derived from a bovine cell source which factor comprises at its N-terminus the amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-.

73. (New) The method of Claim 68 wherein for the isolated and purified bovine endothelial cell growth factor has an N-terminus amino acid sequence of - Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-Val-Val-Lys-Phe-Met-Asp-Val-Tyr-Gln-(Arg)-Ser-Phe-X-Arg-Pro-Ile-Glu-Thr-Leu-(Val)-X-Ile-X-(Gln)-Glu-Tyr-(Pro)- wherein the amino acids in parenthesis are uncertain and the -X indicates an amino acid of unknown identity.

74. (New) The pharmaceutical composition of Claim 68 for promoting the proliferation of bovine endothelial cells in vivo, which composition comprises:

(a) an isolated and purified endothelial cell growth factor wherein

said endothelial cell growth factor is a protein obtained from bovine folliculi stellate cells in the form of a dimeric protein having a molecular weight of approximately 43–45 kd which protein is obtained by SDS PAGE under non-reducing conditions, which protein is administered in an effective amount to human tissue in need of wound healing.

75. (New) The pharmaceutical composition of Claim 74 wherein in subpart (a) the composition further includes a pharmaceutically-acceptable carrier.

76. (New) The pharmaceutical composition of Claim 74 wherein in subpart (a) said protein is present in the composition in between about 25 picrogram/ml and 500 picrogram/ml.

77. (New) The pharmaceutical composition of Claim 74 wherein said purified human

endothelial cell growth factor is formed of two substantially homologous units each having a molecular weight of about 23 kd.

78. (New) The pharmaceutical composition of Claim 76 wherein said protein is derived from a bovine cell source and comprises at its N-terminus the partial amino acid sequence Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-.

79. (New) The pharmaceutical composition of Claim 78 wherein the protein has an N-terminus partial amino acid sequence of <sup>1</sup> Ala-Pro-Met-Ala-Glu-Gly-Gly-Gln-Lys-Pro-His-Glu-Val-Val-Lys-Phe-Met-Asp-Val-Tyr-Gln-(Arg)-Ser-Phe-X-Arg-Pro-Ile-Glu-Thr-Leu-(Val)-X-Ile-X-(Gln)-Glu-Tyr-(Pro)- wherein the amino acids in parenthesis are uncertain and the -X indicates an amino acid of unknown identity.